DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on March 15, 210 has been entered.

Amendment Entry

2. The amendment filed March 15, 2010 has been entered. Claims 1-22 and 29-34 are cancelled. New claims 41-46 have been added. Claims 23-28 and 35-46 are under consideration in this office action.

Withdrawal of Rejections

3. The rejection of claims 23-40 under 35 U.S.C. 112, second paragraph, is withdrawn.

New Grounds of Objection

4. The claims are objected to because of the following informalities: Claim 41 recites "....reacting with blood *in vivo*..." However it is unclear what the blood *in vivo* is referring to. The testing of the substance does not occur *in vivo* based upon the recited

method steps. Moreover, none of the recited method steps occur *in vivo*. Therefore the *in vivo* reference appears to be informality and appropriate correction is required. If however, the "blood *in vivo*" is referring to something specifically, then appropriate clarification is required.

New grounds of Rejection

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 23, 25, 26, 28, 41, 43, 44 and 46 are rejected under 35 U.S.C. 102(b) as being anticipated by Allen et al., (Cryobiology. 1978. Vol. 15:375-381).

Claim 23 is drawn to a method for testing blood for reaction to a substance comprising the steps of: selecting a cryopreserved unit dose of a blood product and a cryopreservative from among a plurality of identical cryopreserved unit doses obtained from a single or pooled sample of blood taken from a human or animal; thawing the cryopreserved unit dose; contacting the thawed, cryopreserved unit dose with the substance; and determining, by biological, physical, chemical, or physicochemical means, whether the unit dose reacts with the substance in an immunofunctional, toxic, or modulatory blood reaction.

Claim 41 is drawn to an *in vitro* method for testing a substance for potentially reacting with blood *in vivo* comprising the steps of: selecting a cryopreserved unit dose of a blood product and a cryopreservative from a collection of multiple identical cryopreserved standardized unit doses obtained from a single or pooled sample of blood taken from a human or animal; thawing the cryopreserved unit dose; contacting the thawed, cryopreserved unit dose with the substance; and determining, by biological, physical, chemical, or physicochemical means, whether leukocytes in the unit dose react with the substance in an immunofunctional, toxic, or modulatory blood reaction.

Allen et al., disclose post-thaw suspension of red cell cryopreserved with hydroxyethyl starch (HES). Allen et al., determined the nature of red cell damage when different solutions are used to suspend thawed cells (page 375, col. 2).
Allen et al., disclose full units of whole blood collected in CPD (page 375, col. 2).
Allen et al., disclose red cell freezing was done with 30 ml units frozen in liquid nitrogen and thawed in water (47-49°C) as previously described (page 375, col. 2). The hydroxethyl starch, a cryopreservant, was prepared and added in the freezing mixture (page 375, col.2). Allen et al., disclose suspension of cells was done immediately following thaw by mixing equal volumes of the thawed HES-red cell mixture with the suspension solution. The suspension solutions used included saline (0.9% NaCl), plasma, 6% glucose (0.33 M dextrose in water), and buffered dextrose-mannitol (0.18 M dextrose and 0.25 M mannitol in 0.15 M sodium phosphate, pH 7.3). Dextrose (0.2%) in 0.8% NaCl was substituted at times for 0.9% NaCl (page 376, col.1). The multiple suspension solutions are the substances contacted with the thawed cryopreserved

units, see Table 1 (page 376). Allen et al., disclose the parameters used to evaluate the cells at post-thaw and following suspension included cell recovery, saline stability, hematocrit, suspension time, and supernatant hemoglobin levels (page 376, col.1). Electron microscopy was used to determine the viable cells reaction with the suspension substances for toxic or modulatory blood reactions (page 376, col.2).

Therefore, Allen et al., teach the instant claims.

Claim Rejections - 35 USC § 102

6. Claims 23-24, 26-27 and 35-46 are rejected under 35 U.S.C. 102(b) as being anticipated by Lionetti et al., (US Patent 4,004,975 published Jan. 1977)..

Lionetti et al., disclose a method preservation of granulocytes and, more particularly, the freezing of granulocytes of peripheral blood and the production of a substantial yield of thawed viable cells as determined by in vitro characteristics (col. 1, lines 5-10). Lionetti et al., disclose approximately one-half of the leukocytes from whole blood collected in blood banks is readily obtained by centrifugation and sedimentation, nearly all of the leukocytes from millions of blood collections are presently wasted (col. 1, lines 21-25). From banked blood, the utility of such a freezing and thawing system is presently limited, although the potential exists for salvaging about half of the white cells from more than 20 million blood collections in the United States every year. The yields from single units are too low for transfusions into granulocytopenic patients, and pooling of white cells from multiple units is impractical due to immunological differences (col. 1, lines 53-57).

FIG. 1 presents one embodiment of the present invention that features a bag method of isolating granulocytes (leukocytes) which lends itself to sterile handling and which is compatible with the isolating of all of the cellular elements of the blood. In FIG. 1, a preferred bag system is set forth for the cryopreservation of leukocytes. The figure shows bags which are numbered and/or captioned for identification and are provided with tubes 1 and ports 2, respectively. A blood collection bag is indicated at 3 and is attached serially to a white cell sedimentation and freezing bag indicated at 4, and to platelet and plasma bags indicated at 5 and 6, respectively. The flow diagram of FIG. 1 also includes the sequence of operations leading to the separation of red cells, white cells, platelets and plasma, and the freezing, thawing and washing of white cells. Freezing of the white cells is accomplished with a combination of HES, which functions as both a sedimenting and cryoprotective agent, and DMSO, a cryoprotective agent (col. 3, lines 28-52).

Lionetti et al., disclose units of whole blood were used to isolate buffy coat leukocytes (col. 3, lines 55-68). Lionetti et al., disclose the addition of the cryoprotectant, HES and the cyropreservation of concentrated granulocytes and freezing at -80°C (col. 4, lines 1-17 and 50-68). Lionetti et al., disclose the thawing of the granulocytes (col. 5, lines 1-17). Lionetti et al., disclose the leukocytes from the post-thaw concentrates were analyzed by determining different counts, tested for myeloperoxidase activity and the use of a multchannel cell size analyzer (col. 5, lines 20-37). Lionetti et al., teach the inhibition of bacterial growth by granulocytes (col. 5-6, lines 40-21). To measure the effects of frozen granulocytes on growth, *E. coli* were

diluted with 10% TC in Normosol. Thawed granulocytes, isolated by the sedimentation procedure with HES were diluted to 15.0 ml with Normosol. They were then counted electronically by integration of the area under the size distribution curve, diluted to give the desired concentration and added to proliferating *E. coli*. The mixture was incubated and assessed. A group of control experiments indicated that growth was not inhibited by Normosol, DMSO or supernatants from centrifuged fresh and frozen-thawed white cells after mixing with E. coli in exponential growth (col. 6, lines 1-21). The ability of frozen and thawed granulocytes to inhibit growth of *E. coli* was tested by adding thawed leukocytes to actively growing cultures (col. 9, lines 42-43). Photographs of white cells removed from the incubations with *E. coli* revealed ingested bacteria within the phagocytes, not shown (col. 9, lines 63-65). Application of the growth inhibition test to various samples of frozen white cells clearly demonstrates its ability to distinguish viable post thawed cells from those which had undergone cryoinjury (col. 9, lines 65-68). Growth curves of *E. coli* exposed to white cells frozen under optimum conditions were compared with those with another aliquot of cells deliberately subject to conditions which produced thawed cells with low myeloperoxidase activity and high trypan blue staining, with the results shown in FIG. 6 which illustrates the inhibition of growth of *E. coli* by cryopreserved granulocytes.

Lionetti et al., disclose numerous physiological characteristics can be assessed and when taken together have been relied on to help evolve a satisfactory method to concentrate and cryopreserve buffy coat granulocytes (col. 10, lines 45-49). To this end, cell size distributions were made to rapidly screen for morphological change during

manipulations of white cells. Volume changes and fragmentation are easily assessed this way. Trypan-blue dye exclusion was used to enumerate intactness and cryoplasmic membrane characteristics of thawed granulocytes (col. 10, lines 515-55). Myeloperoxidase was employed as a criterion of cytoplasmic granularity; the thesis being that adequately frozen granulocytes should retain an enzyme function related to the microbicidal phase of phagocytosis (col. 10, lines 55-59). Inhibition of growth of *E. coli* also was studied as a means to describe the capacity of cryopreserved granulocytes to undergo the ingestion aspect of phagocytosis (col. 10, lines 59-63).

Therefore, Lionetti et al., disclose the instant claims.

Previous Grounds of Rejection

7. The rejection of claims 23-40 under 35 U.S.C. 102(b) as being anticipated by Livesey et al., (US Patent 5,364,756 published Nov. 15, 1994) is maintained for reasons already of record.

Response to Arguments

8. Applicant's arguments filed March 15, 2010 have been fully considered but they are not persuasive.

Applicant argue that Livesey discloses freeze drying of cryopreserved blood cells followed by reconstitution and thus does not thaw the cells. Contrary to applicants assertion, Livesey et al., disclose in order to maximize stability and satisfy regulatory requirements for the preparation of dried pharmaceuticals or reagents, it is essential

that the residual moisture content. Livesey et al., disclose a heating method wherein the dried product is heated (col. 19, lines 14-22). Livesey et al., disclose at Example 2 entitled preservation and storage of mammalian culture cells beginning at step 5, the dry cells where stored at 4°C. In Step 6, the dry sample was allowed to come to room temperature and half of the sample was rehydrated. Therefore the sample went from 4°C to room temperature which is generally known to be about 20-25°C. It is the position of the office that thawing occurred when the sample went from 4°C to about 20-25°C. Example 3, Steps 5 and 6 recite the same procedure. Therefore, contrary to applicants assertion, Livesey et al., disclose thawing and rehydration, which thereby meets the limitations of the claims.

Therefore, applicants' argument is not found persasusive and the rejection is maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. The rejection of claims 23-40 under 35 U.S.C. 103(a) as being unpatentable over Hill et al., (US Patent 4,731,330 published March 15, 1988) in view of Livesey et al., (US Patent 5,364,756 published Nov. 15, 1994) is maintained for reasons already of record.

The rejection was on the grounds that it would have been prima facie obvious to modify the method for testing blood for reaction to a substance comprising the steps of: - selecting a frozen unit dose of a blood product and from among a plurality of identical unit doses obtained from a single or pooled sample of blood taken from a human or animal; thawing the frozen unit dose; contacting the thawed unit dose with the substance; and determining, by biological, physical, chemical, or physiochemical means, whether the unit dose reacts with the substance in an immunofunctional, toxic, or modulatory blood reaction as taught by Hill et al., wherein the modification incorporates the cryopreservation techniques as taught by Livesey et al., in order to provide a cryopreserved unit which prevents irreversible damage due to the multiplicity of changes that occur during cooling and quick-freezing.

Response to Arguments

10. Applicant's arguments have been fully considered but they are not persuasive.

Applicants argue that Livesey et al., do not teach thawing the cryopreserved unit and Hill does not cure the deficiency. However, it is the position of the office that Hill et al., teach reconstitution of whole blood control. Livesey et al., teach similar rehydration, however Livesey et al., specifically teach the lyophilized dry sample was allowed to come to room temperature followed by rehydration. Therefore the sample went from 4°C to room temperature which is generally known to be about 20-25°C. It is the position of the office that thawing occurred when the sample went from 4°C to about 20-

25°C. Therefore, contrary to applicants' assertion, Hill et al, in view of Livesey et al., teach thawing and rehydration, which thereby meets the limitations of the claims.

Thus applicants' arguments are not persuasive and the rejection is maintained.

Pertinent Art

11. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Moore et al., (Vox Sanguinis. July 1987 Vol. 53, Issue 1, pages 15-18) disclose fresh human blood was collected in CPD, frozen by either the Meryman or the Valeri high glycerol technique, and stored at -80 degrees C. Later the red cells were thawed and resuspended in either saline-glucose wash solution or an additive solution containing ascorbate-2-phosphate, adenine, glucose (dextrose), mannitol and sodium phosphate (DPG). The cells were later assayed weekly for ATP, 2,3-DPG, pH, P50, glucose utilization and lysis. The additive solution, which meets the limitation of the substance, maintained red cell 2,3-DPG at fresh blood levels for 3 weeks and maintained ATP levels sufficiently well to suggest good red cell viability for 21 days. Using this additive solution would make frozen blood a reasonable source of red cells for emergency needs in both military and civilian blood banking. Reid et al., (Transfusion. Sept. 1993. Vol. 33, Issue9. Pages 709-712) disclose an optimized additive solution was developed for the post thaw preservation of red cells that contained adenine, glucose, disodium phosphate, and citrate buffer. This solution, called AS-17, was compared to AS-3 solution in a clinical trial using 40 subjects (20 in each arm). Fresh-frozen red cells were thawed and subjected to a second period of

storage in either solution for up to 3 weeks at refrigerator temperatures. Both solutions yielded red cells with survival in excess of 75%. The AS-17 solution resulted in improved maintenance of pH, p50, and 2,3 DPG compared to that with AS-3, but both solutions appear adequate for 3 weeks of post thaw storage.

It is noted that Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes (PMN or PML) because of the varying shapes of the nucleus, which is usually lobed into three segments. In common parlance, the term *polymorphonuclear leukocyte* often refers specifically to neutrophil granulocytes.

Conclusion

12. No claims allowed.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ja-Na Hines whose telephone number is 571-272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/JaNa Hines/
Examiner, Art Unit 1645